Journal of Medical Virology 56:138–144 (1998)

False Negativity by an Anti-HIV Assay Kit (IMx 8B32) and Evaluation of Its Replacement (IMx 8C98)

John V. Parry,[1]* Keith R. Perry,[1] Steve Harbour,[1] Christine Burgess,[1] Philip P. Mortimer,[1] Nigel K. Blackburn,[2] and Des Martin[2]

[1]*Hepatitis and Retrovirus Laboratory, Virus Reference Division, Central Public Health Laboratory, London, United Kingdom*
[2]*National Institute for Virology, Sandringham, South Africa*

False negativity in a commercial anti-HIV kit (IMx HIV-1/HIV-2 3rd Generation Plus (code 8B32) was investigated, and the kit that superseded it (IMx HIV-1/HIV-2 III Plus, code 8C98) was evaluated. In a comparison on 574 freshly collected anti-HIV-1-positive specimens, 97.2% were more reactive in 8C98 than in 8B32; 35.5% were more than twice as reactive and 8.5% were more than four times as reactive. In 8B32, the signal from 55 specimens selected because of weak reactivity was enhanced 1.5 to 8.8 times by preliminary heating at 56°C for 30 min. The reactivity of the 55 heated sera was then similar to that of the same specimens tested without heat treatment in the 8C98 assay. Reactivity in 8B32 was also increased in 66 of 76 (at least twofold in 20) randomly chosen anti-HIV-positive serum specimens by the addition of EDTA (10 mM final concentration). One of these specimens was false negative (signal:cutoff (S:CO) ratio 0.76) in 8B32, though its reactivity was restored by addition of EDTA (S:CO ratio 9.54). These findings indicate that the inhibitory effect that originally led to false negative findings in 8B32 was probably due to complement activity, and that the same activity was present in the freshly collected specimens used here to evaluate the replacement IMx anti-HIV assay (8C98). The specimen panel employed to evaluate 8C98 included 1,892 anti-HIV-positive and 779 anti-HIV-negative specimens. There were no false negative reactions. The lowest S:CO ratio observed was 6.2 and only 17 (0.2%) anti-HIV-positive specimens gave ratios less than 10. Nine unreproducible false positive reactions arose, all possibly attributable to specimen carryover by the IMx instrument. The performance of 8C98 was also compared with that of 10 other current anti-HIV kits using 21 sets of seroconversion specimens (127 specimens in total), and five performance assessment panels (92 specimens in total) comprised mostly of single bleeds from recent seroconverters. IMx 8C98 was the second most sensitive assay. We found no evidence that the 8C98 kit was prone to the effect that had given rise to false negative results in its predecessor (8B32). ***J. Med. Virol. 56:138–144, 1998.***

KEY WORDS: false negativity; complement interference; fresh serum effect; HIV seroconversion sensitivity.

INTRODUCTION

The IMx assays (Abbott Laboratories, Chicago, IL) are a popular range of viral diagnostic kits that run on a compact, dedicated benchtop instrument with a throughput of about 20 specimens per hour. In early 1996, this instrument and the associated anti-HIV kit (8B32) were being used by 46 (25.4%) of 230 participants in the U.K. NEQAS performance assessment scheme. At the end of March 1996, however, the 8B32 kit had to be withdrawn from use in U.K. laboratories following reports of false negative results. In the U.K., an extensive retesting program was undertaken [Evans et al., 1997].

The known false negative reactions in 8B32, and some unexpectedly weak positive reactions, occurred after modifications to the assay introduced by Abbott Laboratories in mid-1995, which was intended to improve its ability to detect infections with the rare outlier strains of HIV-1 [Gürtler et al., 1994, 1995; Vanden Haesevelde et al., 1994; Simon et al., 1994]. These modifications were not described in detail in the product insert of the new assay made available to customers. However, the IMx software module required to run the IMx assays was supposedly modified to remove the

Contract grant sponsor: the Medical Devices Agency, London, UK.

*Correspondence to: Dr. John V. Parry, Hepatitis and Retrovirus Laboratory, Virus Reference Division, Central Public Health Laboratory, 61, Colindale Avenue, London NW9 5HT, United Kingdom.

Accepted 3 March 1998

initial 1 in 4 dilution of the specimen in assay diluent; a synthetic peptide probe was incorporated, and a chemical used to control nonspecificity (polyvinylsulfonic acid) was removed (Abbott Laboratories, unpublished). When reports of false negativity in the modified assay emerged, some 6 months after its introduction, the manufacturer advised customers to "discontinue use of [the assay] or evaluate each specimen both undiluted and at a 1:4 dilution" [Abbott Laboratories, 1996]. The manufacturer also suggested that the problem might be associated with high-titer positive samples, and themselves investigated the cause of the false negative reactivity. They now ascribe this to a fresh serum effect requiring an intact complement system in the test sample and concomitantly high titers of antibody to HIV p24 [Anonymous, 1997]. A modified version of the kit (8C98) was introduced into U.K. laboratories in October 1996, following extensive evaluation [Perry et al., 1997; Anonymous, 1997]. It is currently being used by 21 (9.2%) of 228 participants in the U.K. NEQAS scheme (July 1997), apparently without mishap.

We report our own investigation of the origin of false negative and unusually weak reactivity in the 8B32 kit and summarize our evaluation of the kit, 8C98, that superseded it. We also compare the sensitivity of the 8C98 kit with 10 other current assays for anti-HIV.

MATERIALS AND METHODS

Specimens

The serum specimens used in this study are described separately below for each component of the investigation. Those for which anti-HIV status is specified were previously screened by enzyme immunoassay (EIA) and were either found to be unreactive, or reactive and had their reactivity confirmed as anti-HIV-1 or -2 positive by supplementary testing. Anti-HIV-negative specimens were collected mostly from U.K. blood donors but also included some specimens from attenders of a same-day testing center. Anti-HIV-positive specimens were drawn from diverse U.K. risk groups, including those likely to have been infected with strains prevalent in Africa, and from a large number of fresh-frozen serum specimens from South Africa.

Kits

Abbott Laboratories provided, free of charge, a single batch of the withdrawn kit, IMx HIV-1/HIV-2 Plus (8B32), which had been given an extended expiration date by the manufacturer, within which time the investigations were completed; also two different batches of the modified kit, IMx HIV-1/HIV-2 III Plus (8C98). Comparative performance data for 10 other currently available anti-HIV-1/2 assays shown in this report were generated in the course of the Public Health Laboratory Service (PHLS)/Medical Devices Agency Evaluation Program based at the PHLS Virus Reference Division.

Both versions of the IMx assay are qualitative microparticle enzyme immunoassays, described by the manufacturer as "three-step sandwich assays." Apart from initial pipetting of specimens and controls into the sample well of each disposable reaction tray, the entire procedure is managed automatically by the IMx system and cannot be monitored visually by the operator. The assay requires a minimum of 150 μl of each serum/plasma specimen. Recombinant HIV-1 (*env* and *gag*) and HIV-2 (*env*) antigens are coated on the microparticle solid phase. HIV-1-O-derived antigens appear not to be incorporated. The IMx probe/electrode assembly mixes the coated microparticles with an aliquot of the specimen in the preincubation well of the reaction tray. A portion of the mixture is transferred to the glass fiber matrix of the tray, which traps the microparticles. Biotinylated recombinant antigens derived from HIV-1 and -2 are then added. These complex with the anti-HIV that, if present, was bound at an earlier stage to the antigens on the microparticles. Unbound substances are washed away through the matrix and then a signal is generated by sequential incubation of the microparticles with antibiotin/alkaline phosphatase conjugate and 4-methylumbelliferyl phosphate, giving rise to a fluorescent product detected by the IMx optical assembly.

Two IMx processors were installed by Abbott Laboratories, who trained our personnel in the use and maintenance of the IMx System. Because of the number of high-titer specimens we were intending to examine, the manufacturer advised us to carry out washes of the probe in the IMx processor between runs (normally only done once a day) to minimize carryover problems, and this procedure was followed.

Comparison of Withdrawn Kit (8B32) With Its Replacement (8C98)

To compare performance, 574 fresh-frozen anti-HIV-1-positive serum specimens were examined by the IMx HIV-1/HIV-2 Plus (8B32) kit and the IMx HIV-1/HIV-2 III Plus (8C98) kit. Fresh-frozen specimens were employed to conserve complement activity. One hundred and twenty-six anti-HIV negative serum specimens (from blood donors) were also tested by both kits, interspersed among the positive specimens. The comparative tests were done by setting up simultaneously runs of the same specimens with both the 8B32 and 8C98 assay versions, one carousel on each IMx system, employing the matched IMx assay programs. This allowed it to be determined whether the interfering effect that had led to false negativity in routine use of the defective assay (8B32) was present in these fresh-frozen specimens. It also permitted investigation into whether the modified assay (8C98) was resistant to the same interference.

There were three specimens that had previously been found to be false negative in the IMx 8B32 assay, and one that had been weakly reactive, with sufficient volume remaining to allow testing by the 8C98 assay.

Only one of these was sufficient to test again by 8B32 to see whether the inhibitory effect was still present.

Investigation of Interfering Effect Associated With 8B32

To test the hypothesis that complement was responsible for interference in the reactivity of the withdrawn assay, 8B32, two further experiments were done. First, a panel of 55 fresh-frozen anti-HIV-1-positive serum specimens identified in the course of the comparison of the two kits as giving weaker reactions in the withdrawn assay than in the modified assay was tested in the withdrawn assay (8B32) with and without preliminary heat treatment at 56°C for 30 min to abolish complement activity. As this experiment depleted most specimens that showed a substantial difference in reactivity between the 8B32 and 8C98 kits, a random selection of 76 fresh-frozen anti-HIV-1-positive specimens was employed in the second experiment to compare reactivity in the withdrawn assay (8B32) with and without EDTA being added directly to the specimens to a final concentration of 10 mM. (EDTA chelates the calcium and magnesium ions necessary for activation of the complement pathway.) Of the 76 specimens, the available volumes allowed only 15 to be examined also by 8C98.

Evaluation of IMx HIV-1/HIV-2 III Plus (8C98) Kit

The evaluation of the IMx HIV-1/HIV-2 III Plus (8C98) kit was carried out prior to release of the product on the U.K. market. A total of 2,898 specimens were used to evaluate the sensitivity and specificity of the IMx HIV-1/HIV-2 III Plus (8C98) kit. Sensitivity was assessed by examining 1,892 anti-HIV-positive specimens, including 1,370 sera from South Africa and 22 sera from London laboratories that had been frozen within a few hours of collection and kept frozen until immediately before testing (574 were tested by both 8C98 and 8B38). Other, nonfresh, U.K. specimens were drawn from various risk categories, and five anti-HIV-2- and one anti-HIV-1-O-positive specimens were also included. Twenty-one commercial seroconversion panels (127 specimens; panels PRB 912, 914, 916–920M, 922–935) and five performance panels (100 specimens; PRB 105, 202, 203, PRZ 202, PRA 202) were employed (all obtained from Boston Biomedica Inc., West Bridgewater, MA). The specificity of the 8C98 kit was assessed by the inclusion of 779 serum specimens from blood donors and other healthy adults.

Throughout the evaluation, the carousels used in the processor were each loaded with mixtures of positive and negative specimens to monitor carryover between tests on positive and negative specimens. The Abbott Mode 1 calibrator, HIV-1-positive, HIV-2-positive, and negative controls were included in each assay run. Specimens that gave rise to qualitatively inaccurate results by the IMx HIV-1/HIV-2 III Plus (8C98) kit were retested in duplicate whenever possible.

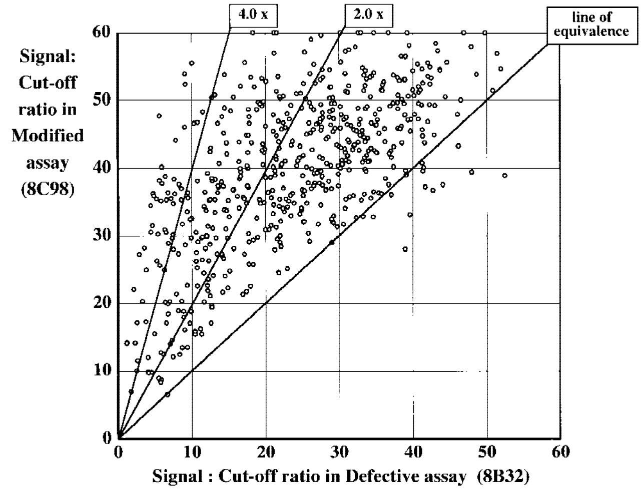

Fig. 1. The reactivity of 574 freshly frozen anti-HIV-positive serum specimens in the defective IMx anti-HIV assay (8B32) and the modified assay (8C98).

RESULTS

Comparison of 8B32 With 8C98

Of the 574 fresh-frozen anti-HIV-positive serum specimens, only 16 (2.8%) gave stronger reactions (though still positive) by 8B32 than by 8C98; the remainder gave weaker ones (Fig. 1). Two hundred and four specimens (35.5%) were more than twice as reactive by 8C98 than by 8B32, of which 49 (8.5%) were more than 4 times as reactive. Three specimens were more than 10 times as reactive. Whereas in 8B32 88 (15.3%) of these specimens gave signal:cutoff (S:CO) ratios of less than 10.0, in 8C98 there were only eight that did so (1.4%). None of the 126 anti-HIV-negative specimens were reactive by the 8B32 assay, but four were by 8C98.

All four of the samples that had been false negative previously (3) or unusually weakly reactive (1) in 8B32 were clearly reactive in 8C98 (S:CO ratios 10.6; 10.8; 13.1; 9.3). However, the only originally false negative sample that was available in sufficient volume to be retested by 8B32 at the time of this evaluation had become weakly reactive by that assay (S:CO ratio 3.2).

Of 635 anti-HIV-1-positive serum specimens (including the 51 specimens examined only in the investigation of the effect of EDTA on 8B32 reactivity, see below), one (0.2%) alone was false negative in 8B32, though 44 (6.9%) others were less reactive than the weakest reaction among 1,892 anti-HIV-1-positive specimens tested by 8C98.

Investigation of Interfering Effect Associated With 8B32

The 55 anti-HIV-positive sera that had been selected as more weakly reactive by 8B32 than by 8C98 became

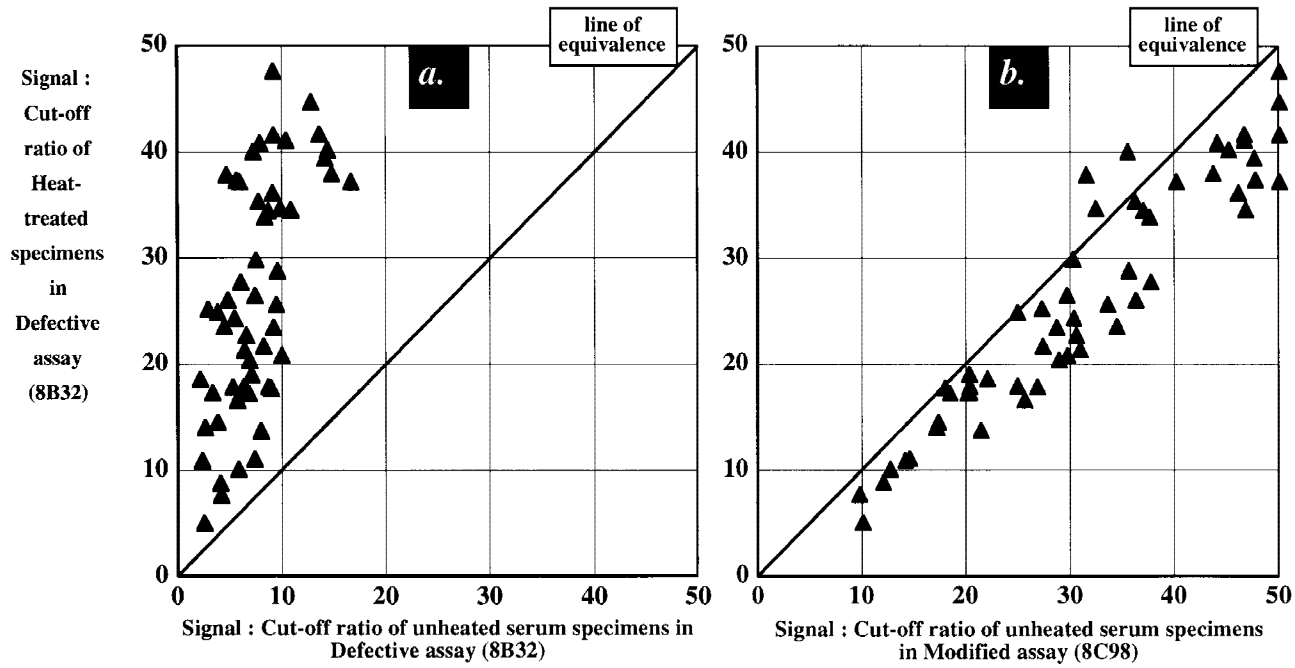

Fig. 2. **a:** The effect of heat treatment (56°C for 30 min) on the reactivity of 55 fresh-frozen serum specimens in the defective IMx anti-HIV assay (8B32). The specimens were selected because they were weakly reactive in 8B32. **b:** The reactivity of the same specimens as in 2a, when tested after heat treatment in the defective assay (8B32) compared with their reactivity untreated in the modified IMx anti-HIV assay (8C98).

more reactive in 8B32 once heated at 56°C for 30 min (Fig. 2a). The ratios of reactivity obtained in 8B32 with and without heating ranged from 1.52 to 8.83 (median 3.50). Fifty-one (93%) specimens were more than twice, 33 (60%) more than four times, and six (11%) more than six times as reactive by 8B32 following heat treatment.

The reactivity of the heated specimens by 8B32 correlated closely with that of the same specimens tested untreated by 8C98 (Fig. 2b). Reactivity when employing the S:CO ratio was generally a little greater by the new assay (8C98), but the ratios of these values for the two assays (8C98:8B32 (heated)) ranged only between 0.83 and 1.97 (median 1.21).

The effect of EDTA on the fresh-frozen anti-HIV-1-positive specimens was less striking than that of heating (Fig. 3a). Overall, 20 (26%) specimens had an increased reactivity of more than twofold in the presence of EDTA. Of the 76 specimens, 12 gave rise to S:CO ratios of <10.0 when tested in the absence of EDTA, including one that was unreactive (S:CO ratio 0.76). Of the 12 weakly reactive specimens, all but one showed a greater than twofold increase in reactivity in the presence of EDTA; seven increased in reactivity more than fourfold. The largest increase, which was more than 13-fold, was seen with the specimen that had been false negative. Unfortunately, only 15 of the 76 EDTA-treated serum specimens were of sufficient volume also to be tested without added EDTA in the modified assay (8C98). The findings showed that EDTA treatment of serum specimens increased reactivity by 8B32 to levels approaching those when the same specimens were tested without treatment by 8C98 (Fig. 3b).

Evaluation of 8C98: Performance on Anti-HIV-Positive and Negative Specimens

All 1,892 anti-HIV-positive specimens were reactive by 8C98; the weakest S:CO ratio was 6.2 and only 17 (0.9%) specimens gave S:CO ratios less than 10. The 8C98 kit discriminated well between positive and negative specimens, with 1,758 (92.9%) positive specimens giving signals greater than 20 times the cutoff and no negative specimen signaling more than 1.4 times the cutoff (Fig. 4).

Of the 779 anti-HIV-negative specimens included in the panel, nine (1.3%) were reactive when initially tested by IMx 8C98, but none of the nine were reactive when retested. The nine unrepeatable positive reactions may be attributable to carryover contamination of them with material from adjacent positive specimens. This might arise if the automatic cleaning of the IMx processor probe between its pipetting of each specimen was not wholly efficient.

Evaluation of 8C98: Comparison With Current Anti-HIV Assays

The performance of IMx 8C98 on seroconversion and other panels of weakly reactive sera was good compared with that of 10 other current commercial kits (Table I). One only, the Sanofi Diagnostics Pasteur Genscreen HIV 1/2 (code 72277), performed better than 8C98, but the small difference is unlikely to confer clinical advantage. There was, in fact, very little differ-

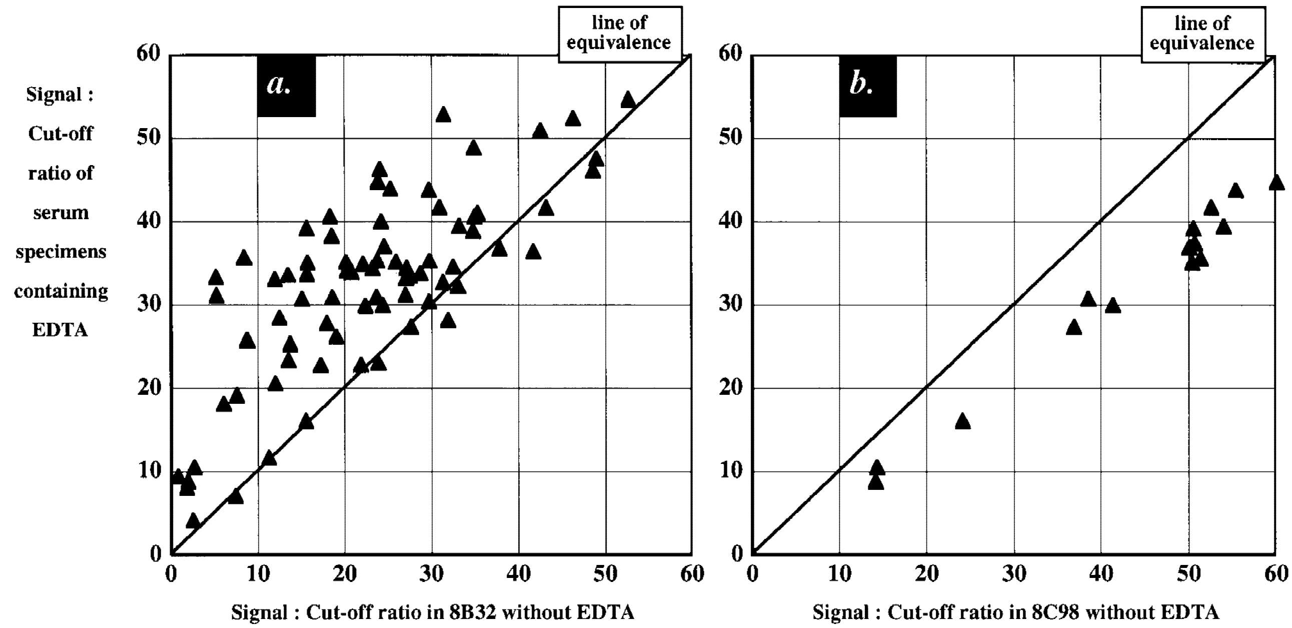

Fig. 3. **a:** The reactivity in the defective IMx anti-HIV assay (8B32) of 76 random freshly frozen anti-HIV-positive serum specimens, with and without added EDTA (final concentration 10 mM). **b:** The reactivity in IMx 8B32 of 15 of the 76 specimens shown in 3a, to which EDTA had been added plotted against their reactivity without added EDTA in the modified IMx anti-HIV assay (8C98). Note that only 15 were of sufficient volume to allow testing also in 8C98.

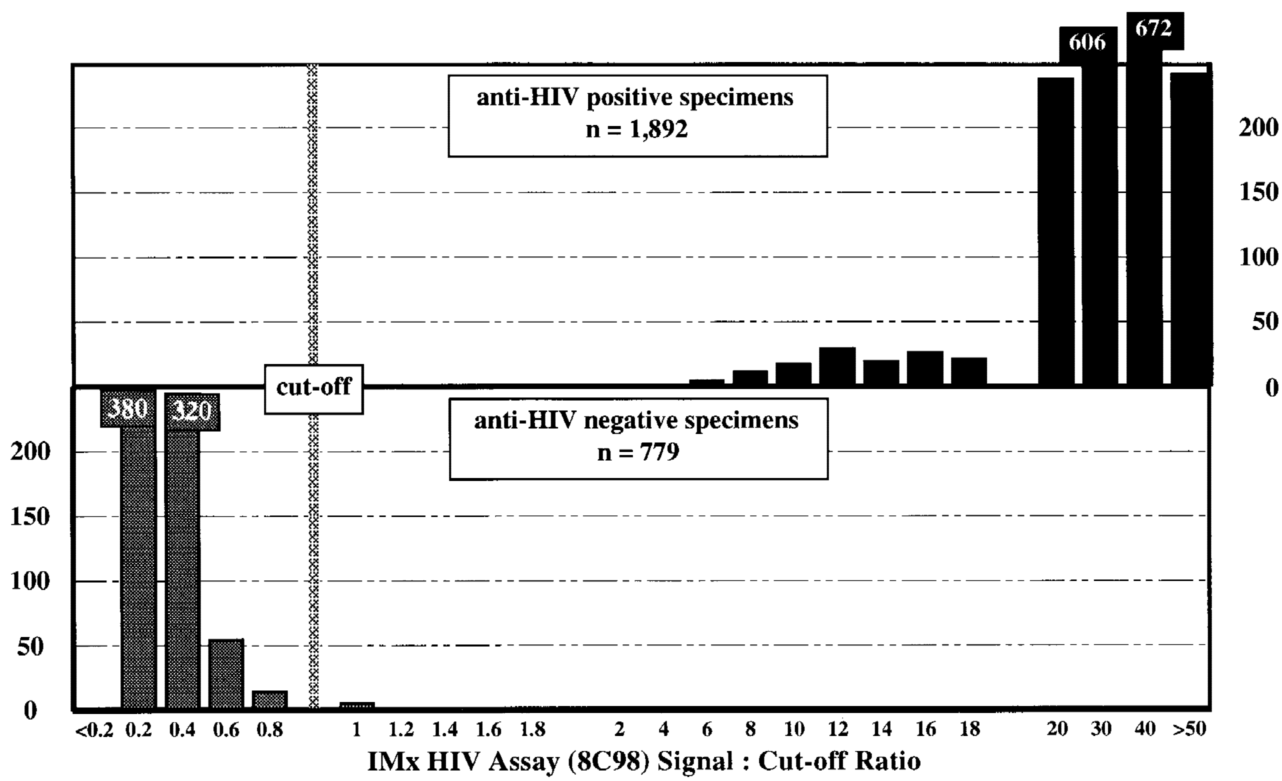

Fig. 4. The distribution of reactivities of anti-HIV-positive and -negative specimens in the modified IMx anti-HIV assay (8C98).

ence between the top-ranked five kits, and only the worst three (Innotest HIV-1/HIV-2 (M422); Clonesystems Detect-HIV (851403); Launch Bioelisa (3000–1106/7)) showed convincing evidence of poorer sensitivity. Further analysis of the findings on the seroconversion panels indicated that these three least sensitive assays might not detect anti-HIV seroconversion until about 1 week after the most sensitive kits.

TABLE I. Anti-HIV Detection in Seroconversion, Low and Mixed Titer Panels by IMx HIV-1/HIV-2 III Plus (8C98) and 10 Other Anti-HIV Assays, Ranked in Order of Sensitivity

Anti-HIV assay	Code number	Seroconversion panels (21) (127 specimens)	Low titer panels (2), mix titer panels (2), combo panel (1) (92 positive specimens)	Total specimens reactive in each assay	Delay in detection of seroconversion	
					Range (days)	Median (days)
Genscreen HIV 1/2	72277	72	91	163	0–37	0
IMx HIV-1/HIV-2 III Plus	8C98	70	92	162	0–9	0
Ortho HIV-1/HIV-2 ELISA	932380	70	92	162	0–9	0
Murex HIV 1+2	VK 84/85	70	91	161	0–37	0
Vironostika HIV Uniform II+0	84018	69	91	160	0–37	0
Murex ICE HIV 1.0.2 EIA	100A	65	91	156	0–37	0
Biotest anti-HIV 1/2	807005	65	90	155	0–37	0
Wellcozyme HIV 1+2	VK54/5	65	89	154	0–37	0
Innotest HIV-1/HIV-2	M422	51	83	134	0–>167	5
Clonesystems Detect-HIV	851403	50	83	133	0–167	7
Launch Bioelisa HIV	3000 1106/7	43	79	122	0–>167	7

DISCUSSION

The effective withdrawal from the market in March 1996 of one of the most widely used anti-HIV kits, on account of false negative findings, disrupted diagnostic services in the U.K. and elsewhere. The kit had given rise to an unknown number of false negative reports, probably only a few of which were recognized. Seven false negative findings were identified within the U.K., including four that came to light during an extensive retesting program [Evans et al., 1997]. The recognition of false negative results that are due to a specific but unforeseen kit defect is difficult. In this case, the problem was first identified in the U.K. because a PHLS laboratory employed more than one anti-HIV screening test to examine specimens from subjects at increased risk of HIV infection [Dr. G. Underhill, personal communication]. False negative results might sometimes be identified when a patient presents with clinical and risk features consistent with HIV disease, but HIV-infected individuals are usually asymptomatic for most of the duration of their infection. Moreover, they may not disclose risk factors, or they may not belong to one of the groups known to be at increased risk of HIV infection. Under all these circumstances, false negative anti-HIV results are likely to go unrecognized. Some false negative results are probably due to technical or clerical error, but others are due to a specific kit defect, as in this instance. There are also reports of rare cases of individuals who are infected with seemingly unremarkable strains of HIV, but who make little or no antibody response to HIV in many or all anti-HIV assays employed to investigate them [Reimer et al., 1996; Dr. P. Rice, personal communication].

Comparison of the reactivity of fresh-frozen anti-HIV-positive serum specimens in the defective Abbott IMx assay (8B32) and in its replacement showed much weaker reactivity overall in the defective assay, and this allowed us to identify suitable specimens for further investigation of the nature of the inhibitory effect. On the other hand, only one of our specimens was actually false negative in 8B32, an observation not in keeping with the findings of the U.K. patient retesting exercise, in which it was found that the rate of false negative results in the IMx 8B32 assay was about five times higher [Evans et al., 1997]. This difference is probably accounted for by our need to freeze the freshly collected specimens to stabilize them until a sufficiently large number had been assembled for this study. Even a single freeze-thaw cycle may mitigate the inhibitory effect, a conclusion supported by the observation that the single false negative specimen of which there was sufficient residue stored from the U.K. retesting exercise had become reactive in 8B32.

Our investigation of the inhibitory effect in the defective Abbott IMx assay (8B32) showed that the reactivity of nearly all the fresh anti-HIV-positive serum specimens selected was enhanced substantially by heating them at 56°C. The effect of EDTA was less pronounced than heat treatment, but this may mostly be explained by the unselected nature of the specimens examined, which would have included a sizable proportion of specimens not manifesting the inhibitory effect. Nevertheless, it was possible to show that EDTA treatment of serum specimens increased reactivity in 8B32 to levels approaching those when the same specimens were tested without treatment in 8C98. Both heat and EDTA prevent the formation of complement complexes and these findings are in keeping with complement mediating an inhibitory effect on the reactivity of 8B32. They also confirm that a substantial proportion of the anti-HIV-positive specimens that were included in the evaluation panel applied to the modified (8C98) assay did contain the substance that interfered with the reactivity of the defective kit (8B32). Though this effect might be present in many fresh specimens, it was probably sufficiently strong to give rise to a negative result on anti-HIV-positive specimens in only a very few. Indeed, Aguilera Guirao [1997] has reported that in one false negative specimen low dilution (1 in 4) in “normal human plasma” was sufficient alone to abolish the inhibitory effect.

It is impossible to be certain that the replacement kit (8C98) is totally resistant to the complement activity found in every fresh serum, but we know of no evidence that it has given rise to false negative results in 8C98. The findings suggest that it would not be expected to. We understand from Abbott Laboratories that in the 8C98 kit the reaction takes place in the presence of EDTA, which can be expected to inhibit the effect seen with 8B32. The evaluation has also shown that 8C98 is highly sensitive in tests on seroconversion and low titer specimens, and more reactive on a large range of fresh-frozen anti-HIV-positive sera than the assay (8B32) it has superseded.

We note that there were nine unreproducible false positive reactions in tests on 779 negative sera, this despite the manufacturer advising us to perform washes of the probe in the processor between each run (normally recommended only once a day). This shows that the additional maintenance step may not entirely prevent carryover of positive serum on the probe during a run. Carry over will, by its nature, only be a significant problem in laboratories that test high-prevalence populations. It underlines the need to follow published guidance about confirming anti-HIV reactivity by additional tests, including examination of a second specimen [PHLS AIDS Diagnosis Working Group, 1992].

The origin of the damaging and costly failure of the 8B32 assay appears in retrospect to have been a too hasty modification of its protocol in an attempt to increase its sensitivity to antibody to the rare outlier variants of HIV-1 (HIV-1-0). Although the impact of HIV-1-0 infections in most European countries was, and has remained, largely notional, the manufacturer felt impelled in mid-1995 to respond to the clamor for improved sensitivity to them. The expedients adopted may have increased HIV-1-0 sensitivity; however, they also allowed false negative results to occur on specimens from patients infected with the common HIV-1 M strains. If customers had been more realistic in their expectations, and allowed time for the HIV-1-0 problem to be more fully investigated, this unfortunate episode might not have occurred. We do not know whether sensitivity for these very rare infections has now been increased, but we are confident that the revised IMx anti-HIV assay is not prone to false negative results when infections with the predominant clades of HIV-1 are being investigated.

ACKNOWLEDGMENTS

We thank colleagues who provided us with specimens used in the evaluation, particularly Dr. François Simon (Hôpital Bichat, Claude Bernard, Paris) for gifts of several HIV-2- and HIV-1-O-positive specimens and Dr. Mark Zuckerman (PHL Dulwich, London), Professor Don Jeffries (St Bartholomew's Hospital, London), and Dr. Gill Underhill (PHL Portsmouth) who provided the fresh-frozen anti-HIV-positive specimens collected in the U.K. Our thanks are also due to Dr. Marcela Contreras and the staff of the North London Blood Centre for allowing us access to serum specimens from blood donors. Evaluation of 8C98 was in part funded by the Medical Devices Agency.

REFERENCES

Abbott Laboratories (1996): Letter to customers. Location: Abbott Laboratories Maidenhead, Berkshire, U.K., March 29.

Aguilera Guirao A, Cabarcos Ortiz De Barron A, Garcia Riestra C, Reguerio Garcia BJ (1997): Evaluation of IMx HIV-1/HIV-2 III Plus. In Michel G (ed): IMx International Field Evaluation—HIV-1/HIV-2 III Plus." Abbott Diagnostics Division, Wiesbaden, Germany: pp 11–15.

Anonymous (1997): IMx HIV-1/HIV-2 III Plus field evaluation studies: Background and summary of key results. In Michel G (ed): IMx International Field Evaluation—HIV-1/HIV-2 III Plus." Abbott Diagnostics Division, Wiesbaden, Germany: pp 4–10.

Evans BG, Parry JV, Mortimer PP (1997): HIV antibody assay that gave false negative results: Multicentre collaborative study. British Medical Journal 315:772–774.

Gürtler LG, Hauser PH, Eberle J, von Brunn A, Knapp S, Zekeng L, Tsague JM, Kaptue L (1994): A new subtype of human immunodeficiency virus type 1 (MVP 5180) from Cameroon. Journal of Virology 68:1581–1585.

Gütler LG, Zekeng L, Simon F, Eberle J, Tsague JM, Kaptue L, Brust S, Knapp S (1995): Reactivity of five anti-HIV-1 subtype O specimens with six different anti-HIV screening ELISAs and three immunoblots. Journal of Virological Methods 51:177–184.

Perry KR, Harbour S, Burgess C, O'Hara K, Parry JV, Mortimer PP, Blackburn N, Martin D, Garden P, Dhell J (1997): IMx HIV-1/HIV-2 III Plus (product code: 8C98). Medical Devices Agency Evaluation Report #MDA/97/57. HMSO Norwick, Norfolk, UK.

PHLS AIDS Diagnosis Working Group (1992): Towards error-free HIV diagnosis: Notes on laboratory practice. PHLS Microbiology Digest 9:61–64.

Reimer L, Brokopp C, Mottice S, Den R, Nichols C (1996): Persistent lack of detectable HIV-1 antibody in a person with HIV infection—Utah, 1995. Morbidity and Mortality Weekly Report 45:181–185.

Simon F, Ly TD, Bailliou-Beaufils A, Fauveau V, De Saint-Martin J, Loussert-Akaja I, et al. (1994). Sensitivity of screening kits for anti-HIV-1 subtype O antibodies. AIDS 8:1628–1629.

Vanden Haesvelde M, Decourt J-L, De Leys RJ, Vanderborght B, van der Groen G, van Heuverswijn H, Saman E (1994): Genomic cloning and complete sequence analysis of a highly divergent African human immunodeficiency virus isolate. Journal of Virology 68: 1586–1596.